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Both Inherited Susceptibility and Environmental Exposure Determine the Low-Density Lipoprotein-Subfraction Pattern Distribution in Healthy Dutch Families

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Summary

A lipoprotein profile characterized by a predominance of small, dense, low-density lipoprotein (LDL) particles has been associated with an increased risk of atherosclerosis. To investigate whether genetic factors are involved in determining this heavy LDL subfraction pattern, this study was undertaken with the aim of resolving the effects that major genes, multifactorial heritability, and environmental exposures have on the LDL subfraction pattern. In a random sample of 19 healthy Dutch families including 162 individuals, the distribution of the LDL subfraction pattern was determined by density gradient ultracentrifugation. For each subject a specific LDL subfraction profile was observed, characterized by the relative contribution of the three major LDL subfractions—LDL1 (d = 1.030–1.033 g/ml), LDL2 (d = 1.033–1.040 g/ml), and LDL3 (d = 1.040–1.045 g/ml)—to total LDL. A continuous variable, parameter K, was defined to characterize each individual LDL subfraction pattern. Complex segregation analysis of this quantitative trait, under a model which includes a major locus, polygenes, and both common and random environment, was applied to analyze the distribution of the LDL subfraction pattern in these families. The results indicate that the LDL subfraction pattern, described by parameter K, is controlled by a major autosomal, highly penetrant, recessive allele with a population frequency of .19 and an additional multifactorial inheritance component. The penetrance of the more dense LDL subfraction patterns, characterized by values of $K < 0$, was dependent on age, gender, and, in women, on oral contraceptive use and postmenopausal status. Furthermore, multiple regression analysis revealed that approximately 60% of the variation in the LDL subfraction pattern could be accounted for by alterations in age, gender, relative body weight, smoking habits, hormonal status in women, and lipid and lipoprotein levels. In conclusion, our results indicate that genetic influences as well as environmental exposure, sex, age and hormonal status in women are important in determining the distribution of the LDL subfraction patterns in this population and that these influences may contribute to the explanation of familial clustering of coronary heart disease.

Introduction

Several studies have shown that low-density lipoproteins (LDLs) are heterogeneous macromolecules, consisting of discrete subfractions which differ in physicochemical composition (Lee and Alaupovic 1970; Shen et al. 1981; Krauss and Burke 1982; Fisher 1983; La Belle and Krauss 1990; Dormans et al. 1991), metabolic behavior (Teng et al. 1986; de Graaf et al. 1989; Swinkels et al. 1990), and atherogenic potential (de Graaf et al. 1991*a*). Recently, the risk of coronary heart disease (CHD) has been associated with a high prevalence of small, dense LDL particles (Crouse et al. 1985; Krauss 1987; Austin et al. 1988*a*; Kwiterovich

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1988; Swinkels et al. 1989*a;* Campos et al. 1992). It has been shown that LDL heterogeneity is markedly influenced by age, gender, relative body weight, smoking habits, and lipid and lipoprotein levels, together predicting 37% (Swinkels et al. 1989*b*) to 61% (McNamara et al. 1987) of the variability in LDL subfraction patterns. The remainder of the variability has been suggested to be genetically determined. Possible genetic control of LDL heterogeneity was first reported by Fisher et al. (1975), on the basis of molecular-weight measurements of LDL in five families. Also, among patients with hyperapobetalipoproteinemia and familial combined hyperlipoproteinemia, both characterized by a predominance of small, dense LDL particles and an increased risk of atherosclerosis, genetic influences in the etiology have been proposed (Goldstein et al. 1973; Austin et al. 1990*a*). Recently, Austin and Krauss (1986) and Austin et al. (1988*b,* 1990*b*) provided new evidence that the distribution of the LDL subfraction pattern in a community-based healthy population has a genetic base; they distinguished two discrete phenotypes, based on LDL particle size, which were denoted "A" and "B" and which were characterized by a predominance of large and small LDL particles, respectively. Phenotype B appeared to be inherited as a single-gene trait with a dominant mode of inheritance. However, as the distribution of LDL particles, by size, within the LDL density range shows a continuity, the dichotomization of the LDL subfraction patterns into two discrete phenotypes (Austin et al. 1988*b*) may result in considerable overlap between patterns A and B and in a high interindividual variability, in LDL particle size, among subjects with either pattern A or B. In a recent study, we confirmed that a dichotomous classification of the LDL subfraction profile into patterns A and B could indeed not fully reflect the great interindividual variation in LDL subfraction patterns that was observed among 131 healthy subjects when LDL subfractions were detected by density gradient ultracentrifugation (Swinkels et al. 1989*b*); a specific LDL subfraction distribution was found for each subject and was characterized by the relative contribution of the three major LDL subfractions—LDL1, LDL2, and LDL3—to total LDL. Moreover, when family data are useful for genetic counseling, quantitative traits of relatives are often more informative than their affection status. So the LDL subfraction pattern can be considered a quantitative rather than a qualitative trait. When a quantitative trait such as the LDL subfraction pattern is reduced to a dichotomy (LDL subfraction patterns A and B), relevant information is lost. Hence, in the present report we introduced a continuous variable, parameter *K,* to describe each individual LDL subfraction pattern.

The primary aim of this study was to examine in healthy Dutch families the mode of inheritance of the dense LDL subfraction pattern, on the basis of LDL particle density and by means of the quantitative parameter *K*. LDL subfraction patterns were determined among 162 individuals from 19 large kindreds of two generations by density gradient ultracentrifugation. Complex segregation analysis (Morton and MacLean 1974; Lalouel and Morton 1981; Morton 1982; Lalouel et al. 1983; Morton et al. 1983) was then used to investigate the inheritance of the LDL subfraction patterns. To examine possible differences in the information provided by a continuous parameter and a dichotomous classification, complex segregation analysis was carried out using both parameter *K* and a pattern A/pattern B classification based on parameter *K*.

Subjects and Methods

Families

The recruitment of nuclear families took place in The Netherlands. The families were obtained through probands in both the first and second generations and were ascertained by survey among healthy individuals in our department. For families to participate in the study, the inclusion criterion was a minimal family size of six individuals with a minimal age of 16 years.

Nineteen nuclear families with an average of seven or eight children per family were screened, for a total of 179 individuals. For most families, all family members were visited on the same day. All parents lived in the southern and eastern areas of The Netherlands. The children were living all over The Netherlands, within a 2.5-h drive from the city of Nijmegen. Each subject completed a medical history questionnaire used to collect information on those factors that have been associated with variations in lipid and lipoprotein levels, including life style (e.g., profession, alcohol use, cigarette smoking, and physical activity) and hormonal status (pre- or postmenopausal status in women, postmenopausal hormone use, and oral contraceptive [OC] use), medical status (e.g., diabetes, renal impairment, and liver diseases) and medication use. Subjects who were pregnant, had lipid disorders (total cholesterol >6.5 mmol/liter and triglycerides >2.5 mmol/liter) or cardiovascular or other serious

diseases, or used medications which are known to influence serum lipids and lipoproteins levels were excluded from the study. Eight children were abroad at the time of the study. In total, 162 subjects were left, including all parents, who gave their informed consent to participate in this study. From each subject, blood was sampled in evacuated collection tubes (Corvac) after an overnight fast. Serum was isolated within 2 h, for determination of the LDL subfraction pattern and of the lipid, lipoprotein, and apolipoprotein levels. Nonlocal participants were visited at their homes, and blood was transported on ice to the laboratory within 3 h. All participants were Caucasian, and random mating was assumed.

Detection and Analysis of LDL Subfraction Patterns

LDL subfractions were detected by single-spin density gradient ultracentrifugation according to a method described elsewhere (Swinkels et al. 1987). After ultracentrifugation the three major LDL subfractions—buoyant LDL1 (d = 1.030–1.033 g/ml), intermediate LDL2 (d = 1.033–1.040 g/ml), and dense LDL3 (d = 1.040–1.045 g/ml) were visible as three distinct bands in the middle of the ultracentrifugation tube (fig. 1). The tube of each subject was placed in a specially designed rack and was photographed (Swinkels et al. 1989*b*). After densitometric scanning of the slide of the tube with the LDL subfraction patterns in triplicate, different aspects of the densitometric curves, including peak height, peak width, peak area, and distances between peaks, were examined for their ability to describe an individual LDL subfraction pattern. Analysis of the densitometric scans was performed by the LKB 2190 GelScan program on an Apple IIe computer according to a method described elsewhere (Swinkels et al. 1989*b*). As each subject showed her or his own LDL subfraction pattern, characterized by the relative contribution of the three major LDL subfractions to total LDL, all three LDL subfractions were considered in the search to define a continuous variable to quantify the LDL subfraction profile. It appeared that both the relative peak height and relative peak area were most informative in expressing the relative contribution of each subfraction to total LDL. So, initially, two different continuous variables, based on peak heights and peak area (Swinkels et al. 1989*b*), were introduced, to quantify the LDL subfraction patterns. Since similar results were obtained when the distribution of the LDL subfraction pattern quantified by either peak heights or peak area was analyzed, and since both continuous variables showed a strong significant correlation ($r = -.85$, $P < .001$), in the present report the results will be presented for the continuous variable based on peak heights and defined as parameter K.

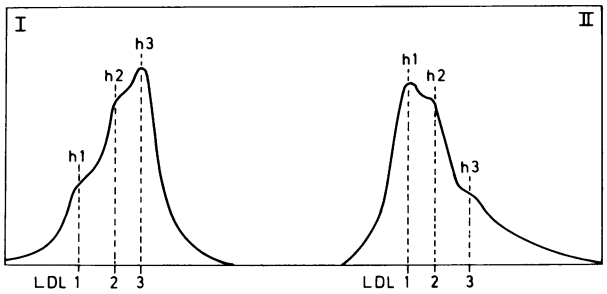

Figure 1 Densitometric scanning patterns of the LDL subfraction distributions, based on density gradient ultracentrifugation of sera. Representative examples of a dense and buoyant LDL subfraction profile are shown. In scan I the highest peak is h3 (= LDL3), reflecting a dense LDL subfraction pattern. In scan II the highest peak is h1 (= LDL1), i.e., a buoyant LDL subfraction pattern. The relative peak heights were used to determine the relative contribution of the three LDL subfractions to total LDL, expressed in the quantitative parameter K. The dense LDL subfraction pattern (h1 − h3 < 0) was characterized by a negative value of K (scan I; K = .22, calculated by K = [%h1 − %h3]/[%h2 − %h1]), whereas a more buoyant LDL subfraction pattern (h1 − h3 > 0) was represented by a positive value of K (scan II; K = .18, calculated by K = [%h1 − %h3]/[%h2 − %h3 + 1]).

To express the relative contribution of each LDL subfraction to total LDL, the mean relative peak heights of the major LDL subfractions on the three scans (total LDL [100%] = LDL1 [%h1] + LDL2 [%h2] + LDL3 [%h3]) (figs. 1 and 2) were used to define parameter K as a continuous variable, to describe each individual LDL subfraction pattern. When a subfraction pattern was characterized by a predominance of buoyant LDL particles (h1 − h3 > 0), K was calculated by K = (%h1 − %h3)/(%h2 − %h3 + 1). So the more buoyant LDL subfraction patterns, mainly consisting of LDL1 and LDL2, were characterized by positive values of K ($0<K<1$). In the case of a predominancy of heavy, dense LDL particles (h1 − h3 < 0), K was calculated by K = (%h1 − %h3)/(%h2 − %h1 + 1), resulting in negative values of K ($-1<K<0$) for the more dense patterns, predominated by LDL2 and LDL3 (figs. 1 and 2). So the values of K varied between −1 and +1; the value of −1 reflects a very heavy LDL subfraction profile consisting of one LDL subfraction—i.e., dense LDL3—whereas the value of +1 reflects a very light LDL subfraction profile characterized by the presence of only very light LDL1. Although some of the information is lost in the

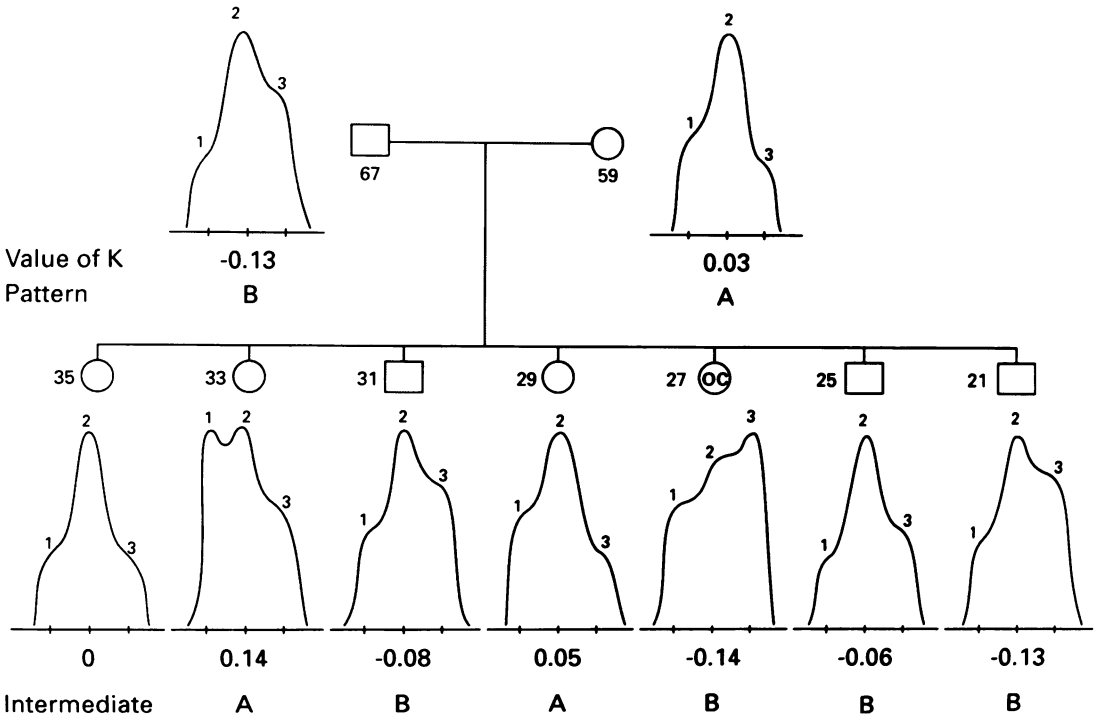

Figure 2 Pedigree of family 19, showing the densitometric scanning patterns of the LDL subfraction profiles obtained after density gradient ultracentrifugation of sera. Each LDL subfraction profile is characterized by a specific value of parameter *K*, calculated as described in the legend of fig. 1. In addition, each LDL subfraction profile is classified as pattern A ($K>0$), pattern B ($K<0$), or the intermediate LDL subfraction pattern ($K=0$). For each individual the age is indicated. 1 = LDL1 (1.030–1.033 g/ml); 2 = LDL2 (1.033–1.040 g/ml); and 3 = LDL3 (1.040–1.045 g/ml).

calculation of parameter *K*, this quantitative measure provides an approximation of each individual LDL subfraction profile, reflecting the great interindividual variety in LDL subfraction patterns. Furthermore, parameter *K* allows the contribution of all three main LDL subfractions to total LDL to be taken into account, instead of only the major LDL subfraction, when the LDL subfraction pattern is defined (McNamara et al. 1987; Austin et al. 1988*a*, 1988*b*; Campos et al. 1988).

To compare the information provided by the continuous parameter *K* with the dichotomous classification as reported in the literature, the LDL subfraction patterns were also subdivided into a light (= A) or heavy (= B) LDL subfraction pattern, by introducing a threshold for parameter *K*. We considered the LDL subfraction profile with a predominant LDL2 peak (h2) and with equal contributions of LDL1 and LDL3 (h1 = h3) to be the intermediate LDL subfraction profile, with the value of *K* being 0 (%h1 – %h3 = 0) (fig. 2). With increasing relative contributions of LDL1, a light LDL subfraction profile was observed, with LDL1 and LDL2 contributing most to total LDL (K = [%h1 – %h3]/[%h2 – %h3 + 1]). The light LDL subfraction pattern A was defined by $K \geqslant 0$. With increasing relative contributions of LDL3, a more heavy LDL subfraction pattern was found, with LDL2 and LDL3 contributing most to total LDL (K = [%h1 – %h3]/[%h2 – %h1 + 1]). So the heavy LDL subfraction pattern B was defined by $K < 0$ (fig. 2).

Plasma Lipid and Lipoprotein Assays

The density of 2.9 ml of serum was raised to d = 1.019 g/ml by the addition of 0.5 ml of a solution containing NaCl (11.42 g/liter), KBr (133.48 g/liter), and EDTA (0.1 g/liter) (d = 1.10 g/ml). Very-low-density lipoprotein (VLDL) + intermediate-density lipoprotein (IDL) were isolated by ultracentrifugation for 16 h at 165,000 *g* in an IEC B-60 fixed-angle rotor 468 (Damon/IEC) (Swinkels et al. 1989*b*). LDL-cholesterol was calculated by subtraction of VLDL + IDL cholesterol and high-density lipoprotein (HDL)–cholesterol from total serum cholesterol. HDL-cholesterol was determined in whole plasma by the

polyethylene glycol 6000 method (Demacker et al. 1980). Serum total cholesterol and triglycerides were determined by enzymatic, commercially available reagents (catalog no. 237574; Boehringer-Mannheim [Germany] and Sera Pak, catalog no. 6639; Miles [Italy], respectively).

Apoprotein B in the $d > 1.019$ g/ml fraction was determined in duplicate on two different plates by radial immunodiffusion in 0.8% (w/v) agarose in barbital buffer, pH 8.6. The 0.8% (w/v) agarose contained 0.3% (v/v) anti-apoB antiserum, raised in rabbits against human LDL (1.030–1.050 g/ml). When duplicates differed by >10%, radial immunodiffusion was repeated (Swinkels et al. 1989*b*).

Complex Segregation Analysis

The segregation of the LDL subfraction pattern in the families was studied using the mixed genetic model for nuclear families, as developed by Morton and MacLean (1974; also see Lalouel and Morton 1981; Morton 1982; Lalouel et al. 1983; Morton et al. 1983). The main purpose of this model is to discriminate a major locus from polygenic and environmental effects. The mixed model assumes that the phenotype, i.e., the LDL subfraction pattern, results from the independent contribution of (1) a major locus, (2) a multifactorial transmissible component (including polygenes and/or common environment), and (3) residual random environmental effects. The major-locus effect results, under a genetic hypothesis, from segregation at a single locus of two alleles, C and c, leading to three genotypic classes (CC, Cc, and cc). The model does not include multiple alleles and two or more major loci (Morton and MacLean 1974). The mixed model allows analysis of both quantitative measurements (parameter *K*) and dichotomous variables (A or B LDL subfraction pattern).

To increase the power of the segregation analysis to reveal genetic influences on the LDL subfraction profile, the LDL subfraction profile (i.e., parameter *K*) variation due to age, sex, and hormonal status was taken into account by introducing liability classes; each individual was assigned to one of five subgroups which were based on age, sex, and hormonal status in women (table 1) and which were introduced into the model as discrete liability classes (Morton et al. 1983). The values of parameter *K* were standardized within each liability class (mean = 0; and SD = 1), to control for the parameter *K* variation due to age, sex, and hormonal status. The segregation analysis was performed on the standardized values of *K*.

To be able to compare our results on the inheritance of the LDL subfraction pattern (parameter *K*) with those in the literature, the dichotomous classification in patterns A and B, based on parameter *K*, was also applied in the segregation analysis, in analogy with the study by Austin et al. (1988*b*). Again, in the distribution of patterns A and B the variation dependent on age, sex, and hormonal status (table 1) was controlled for by introducing discrete liability classes.

The working method of the segregation analysis is to compare the likelihood of different modes of inheritance, both genetic and environmental. This requires the estimation of parameters appropriate to the different models tested. The parameters specifying the major locus include *D*, the degree of dominance at the major locus; *T*, the difference in means between opposite homozygous genotypes, expressed in SD units on the scale of parameter *K*; *Q*, the gene frequency of the major locus; and *T*1, *T*2, and *T*3, the respective probabilities that genotypes CC, Cc, and cc transmit the allele C. Recently, Iselius and Morton reported that only the use of Mendelian transmission probabilities ($T2 = .5$) were valid in the POINTER computer program (Iselius and Morton 1991). So, if the major locus is assumed to be Mendelian, *T*2 is fixed at .5 ($T1 = 1$; and $T3 = 0$). Parameter *H* represents the multifactorial (polygenic and/or cultural) heritability. Maximum-likelihood estimation was used, and the -2 log likelihood values for the corresponding models were compared by a likelihood-ratio (χ^2) test. The results presented are confined to analyses of nuclear families. All calculations were performed using the computer program POINTER (Morton et al. 1983).

Statistical Analysis

Serum triglycerides and VLDL-triglycerides were transformed logarithmically because of skewing of the distributions. Means and SDs of these variables are, however, reported in antilog units, for ease of interpretation.

To determine the difference, in lipid and lipoprotein values, between LDL subfraction patterns A and B, these values were corrected for the influence of gender, age, body mass index (BMI), smoking habits, and OC use and were tested by analysis of covariance. All two variable interaction terms with the LDL subfraction pattern were included in the model. The nonsignificant interactions were deleted. This resulted in the inclusion of the interaction term between OC use and subfraction pattern for VLDL-triglycerides. With the

final model, adjusted lipid and lipoprotein values were estimated for 50-year-old nonsmoking men with a BMI of 24, and the difference between LDL subfraction patterns A and B, in lipid and lipoprotein values, was tested by using analysis of covariance.

Pearson correlation coefficients were computed to determine correlations between parameter K and the variables age, BMI, and smoking. Partial correlation coefficients, controlling for the effect of age, BMI, smoking, and OC use, were computed to determine correlations between parameter K and the variables serum cholesterol, serum triglycerides, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, VLDL-triglycerides, and LDL-apoB.

Multiple linear-regression analysis was performed to determine the influence of gender, age, BMI, smoking habits, and OC use (independent variables) on parameter K (dependent variable). Stepwise multiple linear-regression including forward-selection and backward-elimination procedures was used to examine significant contributions of the independent variables (serum cholesterol, serum triglycerides, LDL-cholesterol, HDL-cholesterol, LDL-apoB, VLDL-cholesterol, and VLDL-triglycerides) to the prediction of parameter K. Any influence of gender, age, smoking habits, BMI, and OC use was taken into account by forcing them in each model. Statistical analysis involved procedures from the Statistical Analysis System (SAS) computer programs (SAS Institute, Cary, NC).

Results

Characteristics of the Families

In total, 162 subjects (80 men and 82 women) participated in the study. Because of a technical error, the LDL subfraction patterns of three subjects (second generation) were not determined. So, in total, 159 subjects (78 men and 81 women) were included in the analysis. The first generation included 19 men and 19 postmenopausal women. None of the postmenopausal women used hormones. The mean age of this first generation was 65.4 ± 7.0 years; the women were significantly younger than the men (63.1 ± 6.4 vs. 67.7 ± 7.0 years, respectively; t-test, $P < .05$). The second generation included 121 subjects (59 men and 62 women). The 62 women were all premenopausal, and 20 (32%) were using low-dose oral contraceptives. The mean age of the second generation was 31.5 ± 7.3 years, with no significant age difference between men and women (30.7 ± 7.3 vs. 32.2 ± 7.2, respectively). For all subjects ($n = 159$), the mean BMI was 22.8 ± 2.3, the men being heavier than the women (23.2 ± 2.0 vs. 22.3 ± 2.5, respectively; t-test, $P < .05$). Of all subjects, 24.5% ($n = 39$) were smokers (1–50 cigarettes per day). Among men there were significantly more smokers than there were among women (32.1% vs. 17.3%; χ^2 test, $P < .05$). All subjects were normolipidemic; none of the subjects reported diseases or use of medications which are known to influence lipid metabolism.

Analysis of the LDL Subfraction Patterns

In all sera, mostly three distinct LDL subfraction bands could be distinguished in the LDL density range; they were separated by a clear interface (fig. 1). For each subject the LDL subfraction pattern was characterized by the relative contribution of the three major LDL subfractions to total LDL, approached by the quantitative parameter K. So the light LDL subfraction patterns were characterized by a major peak of large, buoyant LDL1 or LDL2 and by a minor peak of smaller, denser LDL3, resulting in a positive value of K ($0 \leqslant K < 1$), whereas the heavier, dense LDL subfraction patterns had both a major peak of small, dense LDL3 or LDL2 and skewing of the curve toward the lighter subfractions LDL1, resulting in a negative value of K ($-1 < K < 0$) (figs. 1 and 2).

For the subjects studied, the values of K varied between -1 and $+1$. Among the studied subjects, the frequency distribution of K showed a normal distribution, as presented in figure 3. Furthermore, the distribution of the LDL subfraction patterns appeared to be related to gender, age, and (in women) on hormonal status (table 1). The median value of parameter K was significantly lower in men than in women ($P < .001$ by Wilcoxon's test) and decreased with age, in both sexes. In addition, compared with premenopausal women not taking OCs, women using OCs had a lower median value of K; the LDL subfraction pattern distribution among premenopausal women using OCs resembled that of men and postmenopausal women (table 1). To be able to relate our results on the LDL subfraction pattern distribution, defined by parameter K, to those in the literature, we used parameter K to define patterns A and B, in analogy with other studies (e.g., McNamara et al. 1987; Austin et al. 1988*b*). Our distribution of LDL subfraction patterns A and B, based on parameter K, was strikingly consistent with previously reported results (McNamara et al. 1987; Austin et al. 1988*b*; Campos et al. 1988), even

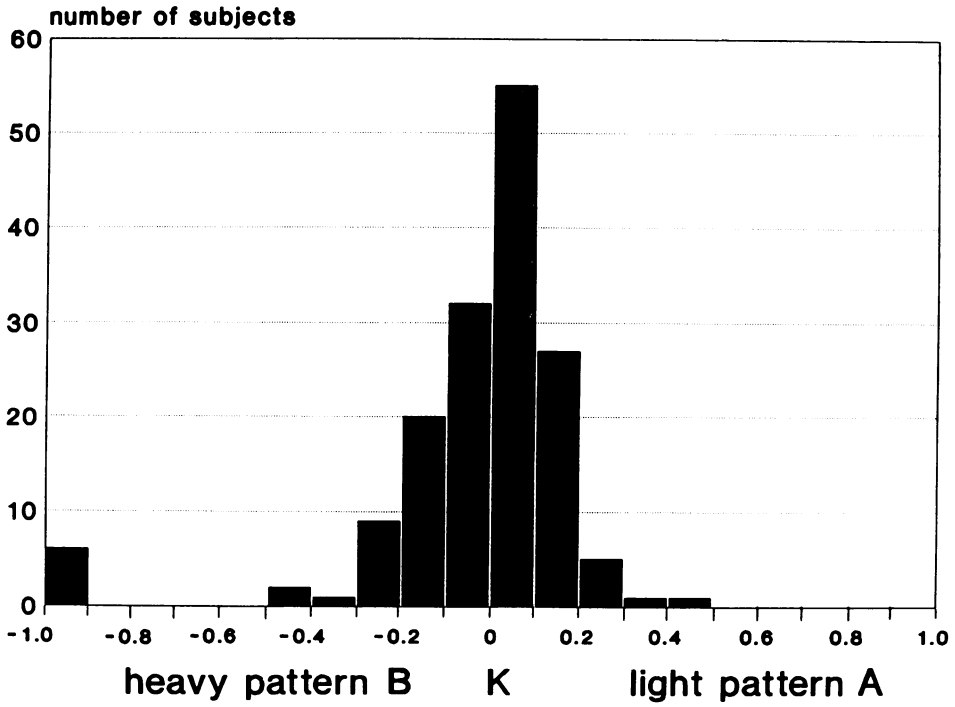

Figure 3 Frequency distribution of parameter K among 159 subjects. The heavy LDL subfraction pattern B was defined by $K < 0$, whereas the light LDL subfraction pattern A was represented by values of $K \geq 0$.

though a different isolation procedure and classification for the LDL subfraction patterns was applied (table 1). These results indicate a strong relationship between parameter K and the previously reported classification in patterns A and B.

Reproducibility of the Quantification of the LDL Subfraction Profile

Each LDL subfraction profile was scanned in triplicate. Within all triplicates the shape of the densitometric curves, including the number of peaks, the peak

Table 1

Distribution of LDL Subfraction Pattern, by Gender, Age, and Hormonal Status

Subjects (n)	Parameter K[a]	LDL Subfraction Pattern B, Determined by[b]		
		Present Study[c]	Austin et al. 1988*b*[d]	McNamara et al. 1987 and Campos et al. 1988[d]
Female (81):	.047 (−.136, .179)	31%	25% (n = 154)	
Premenopausal OC users (20) ...	−.038 (−.192, .130)	55%		
Control (42)	.083 (−.035, .192)	17%	13% (n = 103)	15% (n = 142)
Postmenopausal, age >50 years (19)	.042 (−.143, .207)	37%	49% (n = 51)	30% (n = 43)
Male (78):	−.041 (−.304, .109)	58%	37% (n = 147)	
Age 20–50 years (59)	−.009 (−.287, .142)	51%	44% (n = 112)	44% (n = 138)
Age >50 years (19)	−.126 (−1.000, .106)	79%		

[a] Expressed as median (50th percentile). Data in parentheses are the 10th and 90th percentiles.
[b] LDL subfraction pattern A (%) = 100% − LDL subfraction pattern B (%)
[c] By density gradient ultracentrifugation.
[d] By gradient gel electrophoresis.

height, the peak width, and the distances between peaks, were similar. The mean value of K for the triplicates was $-.035$ ($\pm .234$), $-.023$ ($\pm .211$), and .030 ($\pm .227$), respectively ($n = 159$). The variation between triplicates (i.e., between subjects within the population) was .223, whereas the variation within triplicates (measurement error) was estimated to be .020. Between the triplicates, no systematic deviation in parameter K was found (one-way ANOVA [analysis of variance]; $P = .41$). The sera of 10 subjects were ultracentrifuged in duplicate at the same time, in two different tubes placed in two different ultracentrifuges. Both tubes were photographed separately, and the LDL subfractions were quantitated by densitometric scanning as described. The mean values of parameter K for the duplicates were $-.077$ ($\pm .185$) and $-.079$ ($\pm .179$), respectively. The between- and within-subject variation was .182 and .008, respectively.

To establish the intraindividual variation in the LDL subfraction profile in time—i.e., the variation in the value of parameter K during the year—we determined the LDL subfraction profile of five different subjects on five different occasions ($t = 0$, $t = 1$ mo, $t = 3$ mo, $t = 6$ mo, and $t = 1$ year). The mean values of K on the five different occasions were $-.104$ ($\pm .242$), $-.048$ ($\pm .173$), $-.088$ ($\pm .200$), $-.043$ ($\pm .233$) and $-.033$ ($\pm .237$), respectively. Among the subjects, the variation of K was .210, whereas the variation within subjects was .037. No significant change in the value of parameter K during the year was found (two-way ANOVA; $P = .23$). So the LDL subfraction profile, reflected in the densitometric curve and in the value of parameter K, appeared reproducible on repeated analysis of freshly isolated sera.

Interrelation of the LDL Subfraction Pattern with Anthropometric Measurements

In table 2 the mean age, BMI, and percentage of smokers among all subjects are indicated. The values of parameter K ($n = 159$) correlated significantly with age and BMI, indicating that, with increasing age and relative body weight, a more dense LDL subfraction pattern was present (table 2). Parameter K did not correlate significantly with smoking habits (table 2). Multiple linear-regression analysis of parameter K revealed that age, gender, BMI, smoking habits, and OC use together contributed 22% to the variation of parameter K, i.e., the LDL subfraction pattern. These results indicate that the prevalence of relatively more-dense LDL subfraction profiles was high among male subjects, smokers, and women using OCs and that it increased with age and BMI.

Similar results were obtained when the pattern A/pattern B classification was used; among subjects with a heavy LDL subfraction pattern B, a significantly higher BMI was noticed, compared with subjects with

Table 2

Adjusted Lipid, Lipoprotein, and Apolipoprotein Levels, for All Subjects and as Stratified by Light (=A, $K \geq 0$) and Heavy LDL (=B, $K < 0$) Subfraction Patterns

	All Subjects (n = 159)[a]	Pattern A (n = 92)[a]	Pattern B (n = 67)[a]	Correlation Coefficient (n = 159); P Value[b]	
Age of parents[b]	65.4 ± 7.0	63.1 ± 7.3	67.0 ± 6.5		
Age of children[b]	31.5 ± 7.3	31.3 ± 7.2	31.7 ± 7.4	−.20	<.01
BMI (kg/m^2)[b]	22.8 ± 2.3	22.4 ± 2.3*	23.3 ± 2.2	−.30	<.001
Smoking	24.5%	16.9%	34.3%	−.09	NS
Total cholesterol (mmol/liter)	5.07 ± .12	5.26 ± .15	4.94 ± .13	.01	NS
Triglycerides (mmol/liter)	1.12 ± .05	.98 ± .06*	1.22 ± .05	−.68	<.001
LDL-cholesterol (mmol/liter)	3.19 ± .10	3.33 ± .13	3.10 ± .11	.01	NS
HDL-cholesterol (mmol/liter)	1.18 ± .04	1.34 ± .05*	1.07 ± .04	.37	<.001
VLDL-cholesterol (mmol/liter)	.70 ± .04	.59 ± .04*	.77 ± .04	−.49	<.001
VLDL-triglycerides (mmol/liter)	.69 ± .06	.57 ± .08*	.77 ± .07	−.70	<.001
Apoprotein B (g/liter)	1.38 ± .05	1.34 ± .06	1.41 ± .05	−.15	NS

[a] For age and BMI data are mean ± SD; for the lipid and lipoprotein levels data are mean ± SE. Mean values of lipid and lipoprotein levels are adjusted, with analysis of covariance, to those of a 50-year-old nonsmoking man with BMI = 24 kg/m^2.

[b] For age, BMI, and smoking habits, Pearson correlation coefficients with parameter K are shown; for the lipid and lipoprotein levels, partial correlation coefficients with parameter K (to control for the effect of age, BMI, and smoking habits) are shown. NS = not significant.

* $P < .001$, vs. pattern B.

pattern A. In addition, among subjects with pattern B there were significantly more smokers than there were among subjects with pattern A (table 2).

Interrelation between the LDL Subfraction Pattern and Lipid and Lipoprotein Levels

The lipid and lipoprotein levels were within the normal range in all subjects (table 2). After correction for age, BMI, and smoking, the values of parameter K were found to be negatively correlated with serum triglycerides, VLDL-triglycerides, and VLDL-cholesterol levels and to be positively correlated with HDL-cholesterol levels. So, with decreasing values of parameter K—i.e., among the more dense LDL subfraction profiles—a more atherogenic lipoprotein profile was observed, characterized by higher serum triglyceride levels and lower HDL-cholesterol levels. It was confirmable that the heavy LDL subfraction pattern B was associated with a more atherogenic lipoprotein profile characterized by significantly higher levels of serum triglycerides, VLDL-triglycerides, and VLDL-cholesterol and with lower levels of HDL-cholesterol (table 2).

As previously reported (Swinkels et al. 1989*b*), all lipid and lipoprotein levels showed strong intercorrelations, except for total cholesterol and LDL-cholesterol levels, which did not correlate with HDL-cholesterol levels (data not shown). Furthermore, all lipid and lipoprotein levels were positively correlated ($P < .001$) with age and BMI; only age did not correlate with HDL-cholesterol levels. Because of these interrelations between lipids and lipoproteins, stepwise multiple regression analysis was performed to evaluate which lipids or lipoproteins contributed significantly to the prediction of the LDL subfraction pattern. In most studies in the literature, values of LDL-cholesterol, VLDL-cholesterol, and VLDL-triglycerides either were not available or were not considered. So, to be able to compare our results with those in the literature, the stepwise multiple regression analysis was performed twice, once excluding LDL and VLDL concentrations (model I) and once including values of LDL and VLDL (model II).

Table 3 shows the results of the two different models that included gender, age, BMI, smoking habits, and OC use as covariates. Both backward and forward

Table 3

Regression Coefficients and Levels of Significance for Variables in Models Most Appropriate for Predicting LDL Density Pattern, i.e., Parameter *K*

Variable[a]	Regression Coefficient ± SE (P), under Model I (−VLDL and −LDL)	Regression Coefficient ± SE (P), under Model II (+VLDL and +LDL)
Intercept	.086 ± .142 (.55)	.104 ± .141 (.46)
Sex:		
Group 1	.043 ± .032 (.18)	.035 ± .031 (.26)
Group 2	.010 ± .032 (.76)	−.007 ± .032 (.82)
Group 3	−.033 ± .036 (.36)	−.042 ± .035 (.23)
Age (years)	−.002 ± .001 (.05)	−.002 ± .001 (.02)
BMI (kg/m^2)	−.001 ± .006 (.91)	−.002 ± .006 (.70)
Smoking	.002 ± .002 (.34)	.001 ± .002 (.49)
Total cholesterol (mmol/liter)	.082 ± .015 (0)	.072 ± .015 (0)
LDL-cholesterol (mmol/liter)	. . .[b]	. . .[c]
HDL-cholesterol (mmol/liter)	. . .[c]	. . .[c]
Triglycerides (mmol/liter)	−.431 ± .034 (0)	−.218 ± .105 (.04)
VLDL-cholesterol (mmol/liter)	. . .[b]	.177 ± .087 (.04)
VLDL-triglycerides (mmol/liter)	. . .[b]	−.366 ± .118 (0)
Apoprotein B ($\times 10^3$ mg/liter)	. . .[c]	. . .[c]
R^2	.60	.63

NOTE.—Group 1 = women not using OCs, vs. men; group 2 = women using OCs, vs. men; and group 3 = women using OCs, vs. women not using OCs.

[a] The first five variables are forced into the model.

[b] This variable is not included in the model.

[c] This variable does not contribute significantly to the model ($P > .05$).

Table 4

Results of Complex Segregation Analysis Based on Five Liability Classes and Standardized Continuous Parameter *K*

Model	*H*	*D*	*T*	*Q*	−2 ln L + C	Comparison	χ^2 (df)
1. General mixed model	.11	.06	4.49	.18	248.2		
2. No inheritance of susceptibility	(0)	. . .	. . .	(0)	340.3	2 vs. 1	92.1 (2)*
3. Multifactorial inheritance only	.38	(0)	(0)	(0)	328.9	3 vs. 1	80.7 (3)*
4. General single locus	(0)	.11	4.67	.17	264.9	4 vs. 1	16.7 (1)*
5. Dominant mixed model	.09	(1.0)	3.63	.02	269.5	5 vs. 1	21.3 (1)*
6. Additive mixed model	.09	(.5)	7.25	.02	269.5	6 vs. 1	21.3 (1)*
7. Recessive mixed model	.12	(0)	4.31	.19	249.3	7 vs. 1	1.1 (1)

* $P < .001$.

stepwise analysis revealed similar results. In model I, excluding LDL-cholesterol, VLDL-cholesterol and VLDL-triglyceride levels, the best model for prediction of parameter *K* included total cholesterol and serum triglycerides. No other lipid or lipoprotein level reached the $P = .05$ level of significance. When serum LDL-cholesterol, VLDL-triglycerides, and VLDL-cholesterol levels were included in the model (model II), total cholesterol, VLDL-cholesterol, VLDL-triglycerides, and serum triglycerides appeared to make a significant contribution to the prediction of parameter *K*. Both models were similar in predicting parameter *K* ($R^2 = .60$). So, including VLDL and LDL lipid levels in the model did not provide additional information in the prediction of the LDL subfraction pattern.

Complex Segregation Analysis

An example for the segregation of the LDL subfraction patterns in one family is shown in figure 2. In agreement with the results presented in table 1, a high prevalence of the more dense LDL subfraction profiles (pattern B, or $K < 0$) was found among the male family members, whereas in most women a more light LDL subfraction pattern (pattern A, or $K \geq 0$) was observed; only one woman, who used oral contraceptives, had a very dense LDL subfraction profile.

Complex segregation analysis was carried out using both the continuous variable, parameter *K* (table 4), and the dichotomous classification in LDL subfraction patterns A and B (table 5). To increase the power of the segregation analysis to reveal genetic influences on the LDL subfraction profile, the LDL subfraction pattern variation due to age, sex, and hormonal status, as reported above, was taken into account by introducing five liability classes (table 1); the values of parameter *K* were standardized within each liability class, to control for age, sex, and hormonal status. The segregation of parameter *K* in the families was tested on the standardized values of *K*. Similarly, when the dichotomous classification pattern A/B was applied in the segregation analysis, discrete liability classes were included in the POINTER computer program, to control for age, sex, and hormonal status.

Several hypotheses for genetic transmission of the LDL subfraction pattern in these Dutch families were tested by the POINTER computer program, postulat-

Table 5

Results of Complex Segregation Analysis Based on Four Liability Classes and a Light (Pattern A, $K \geq 0$) or Heavy (Pattern B, $K < 0$) LDL Subfraction Pattern

Model	*H*	*D*	*T*	*Q*	−2 ln L + C	Comparison	χ^2 (df)
1. General mixed model	.001	.48	2.59	.37	130.4		
2. No inheritance of susceptibility	(0)	. . .	. . .	(0)	148.4	2 vs. 1	18.0 (2)*
3. Multifactorial inheritance only	.77	(0)	(0)	(0)	131.1	3 vs. 1	.7 (3)
4. General single locus	(0)	.49	2.59	.37	130.4	4 vs. 1	0 (1)
5. Dominant single locus	(0)	(1.0)	1.64	.37	130.7	5 vs. 4	.3 (1)
6. Additive single locus	(0)	(.5)	2.56	.37	130.4	6 vs. 4	0 (1)
7. Recessive single locus	(0)	(0)	1.69	.82	130.8	7 vs. 4	.4 (1)

* $P < .05$.

ing no familial clustering of the dense LDL subfraction profile beyond that occurring by chance (model 2), familial clustering without the influence of a major gene (model 3), or genetic transmission by various modes of inheritance (models 4–7). The different models (models 1–7) and corresponding maximum-likelihood parameter estimates are presented in tables 4 and 5. The values in parentheses were fixed in accordance with the model being tested. The lower the value of $-2 \ln L + C$, the greater the likelihood, the better the fit of the model.

Results of the complex segregation analysis using parameter K.—When the standardized values of parameter K were used as a continuous variable in the POINTER program, the hypothesis that clustering of low values of K (i.e., heavy LDL subfraction patterns) in families does not exceed that expected to occur by chance (model 2) could be rejected, suggesting a genetic influence on the LDL subfraction pattern (table 4). The transmission models postulating either multifactorial inheritance component only (model 3) or a general single locus (model 4) were also strongly rejected. So both a multifactorial component and a major gene must be included in the model of inheritance, explaining the distribution of the value of K, i.e., the LDL subfraction pattern distribution in our families. Indeed, the general mixed model (model 1), which includes both the multifactorial component H and a major gene, had the lowest $-2 \ln L + C$ value—i.e., the greatest likelihood—thus supporting the data best. For the major locus, both the dominant (model 5) and additive (model 6) mode of inheritance were rejected. When a recessive mode of inheritance was set for the major locus (model 7), the results were consistent with the general mixed model (model 1). So the value of parameter K in our families appeared to be controlled by both a major autosomal recessive gene and a significant multifactorial inheritance component. The frequency of the proposed allele at the major locus that controls the values of parameter K was estimated to be .19.

In the general mixed model, approximately 64% of the transmission variance could be accounted for by the major locus, 11% by multifactorial inheritance, and the rest (25%) by random environmental exposures. The general mixed model and the recessive mixed model (table 4) were used to estimate the genotype-specific penetrance of the more dense LDL subfraction patterns, characterized by values of $K<0$. Both the general and the recessive mixed model revealed similar results (table 6). For subjects with genotype cc, in which c represents the presence of the defective gene causing dense LDL subfraction patterns ($K<0$), the penetrance was 100%, indicating that all subjects with genotype cc will have a value of $K<0$. For subjects with genotype Cc or CC, the probability of expressing a more dense LDL subfraction pattern was dependent on gender, age, and (in women) hormonal status; the risk of having a value of $K < 0$ tended to increase with age, for both sexes, and was higher for men than for women. Furthermore, it appeared that OC use was associated with a high penetrance of the more dense LDL subfraction patterns. For example, in the general mixed model, 65% of the women with genotype Cc and using OCs had a dense LDL subfraction profile ($K<0$), compared with only 21% of the premenopausal women not using OCs (table 6). Note that, even in the absence of the defective c allele (genotype CC), there was still a high probability of values of $K < 0$ (table 6). In the general mixed model, subjects with genotype Cc showed a slightly higher penetrance of the dense LDL subfraction patterns, compared with those with genotype CC, whereas, in the recessive mixed model, genotypes CC and Cc had,

Table 6

Penetrance of Dense LDL Subfraction Profiles, Characterized by Values of $K < 0$, by Liability Class

	Penetrance, for Genotype[a]		
	cc	Cc	CC
General mixed model:			
Male:			
Age 20–50 years	1.0	.61	.44
Age >50 years	1.0	.86	.75
Female:			
Premenopausal:			
OC users	1.0	.65	.48
Not OC users	1.0	.21	.11
Postmenopausal, age >50 years	1.0	.46	.30
Recessive mixed model:			
Male:			
Age 20–50 years	1.0	.49	.49
Age >50 years	1.0	.78	.78
Female:			
Premenopausal:			
OC users	1.0	.53	.53
Not OC users	1.0	.14	.14
Postmenopausal, age >50 years	1.0	.35	.35

[a] Allele c represents the defective allele causing the dense LDL subfraction patterns with values of $K < 0$.

by definition, an identical risk of expressing a dense LDL subfraction pattern.

In table 7, the frequency distribution of the genotypes present in subjects with light ($K \geq 0$) or heavy ($K<0$) LDL subfraction patterns is shown for both the general and recessive mixed model (table 4). For both models, similar results were obtained. Among all subjects with light LDL subfraction patterns ($K \geq 0$), approximately 75% (range 68%–80%) were expected to have genotype CC, and 25% (range 20%–32%) had genotype Cc, whereas genotype cc was not observed (table 7). All subjects with heavy LDL subfraction patterns ($K<0$) had estimated frequencies of genotypes cc, Cc, and CC of approximately 7%, 30%, and 63%, respectively. Only among OC users with heavy LDL subfraction patterns ($K<0$) did the expected frequency of genotypes cc, Cc, and CC differ (20%, 37%, and 43%, respectively, in the general mixed model).

Table 7

Frequency Distribution of Genotype Present in Subjects with Light or Heavy LDL Subfraction Patterns, Characterized by Values of $K \geq 0$ and $K < 0$, Respectively

	Pattern	Frequency, for Genotype		
		cc	Cc	CC
General mixed model:				
Male:				
Age 20–50 years	$K < 0$	.07	.36	.58
	$K \geq 0$	0	.24	.76
Age >50 years	$K < 0$	.04	.33	.63
	$K \geq 0$	0	.20	.80
Female:				
Premenopausal:				
OC users	$K < 0$	.20	.37	.43
	$K \geq 0$	0	.23	.77
Not OC users	$K < 0$	.06	.35	.59
	$K \geq 0$	0	.28	.72
Postmenopausal, age >50 years	$K < 0$	.09	.37	.54
	$K \geq 0$	0	.26	.74
Recessive mixed model:				
Male:				
Age 20–50 years	$K < 0$	.07	.29	.64
	$K \geq 0$	0	.32	.68
Age >50 years	$K < 0$	.04	.30	.65
	$K \geq 0$	0	.32	.68
Female:				
Premenopausal:				
OC users	$K < 0$	.21	.25	.54
	$K \geq 0$	0	.32	.68
Not OC users	$K < 0$	.06	.30	.64
	$K \geq 0$	0	.32	.68
Postmenopausal, age >50 years	$K < 0$	.10	.29	.62
	$K \geq 0$	0	.32	.68

Results of the segregation analysis using the pattern A/pattern B classification. — In table 8, the observed segregation ratios for LDL subfraction patterns A and B, based on parameter K, among the 19 families are shown. In order to be able to compare our results on the inheritance of the LDL subfraction profile (parameter K) with those in the literature, we also applied the pattern A/pattern B classification, based on parameter K, and four liability classes (men and three groups of women—i.e., premenopausal women using OCs, premenopausal women not using OCs, and postmenopausal women) in the segregation analysis, in analogy to the procedure of Austin et al. (1988*b*). The results are shown in table 5. Whereas the model of no inheritance (model 2) could be firmly rejected, discrimination among the other models was not possible at the $P < .05$ level. Most likely, since there is less power in using a dichtomous trait for the segregation analysis, the sample size in the present study was not large enough to discriminate between complex models of inheritance (i.e., models 3–7).

Discussion

The results of the present study show that the distribution of the LDL subfraction pattern in a random sample of Dutch families has a genetic base; a common, highly penetrant, major autosomal recessive gene, with a population frequency of .19, and an additional multifactorial inheritance component best explain the clustering of the dense LDL subfraction patterns in this population. Several studies have suggested a possible genetic control of LDL heterogeneity, using a variety of techniques to detect LDL heterogeneity and to analyze its genetic susceptibility (Goldstein et al. 1973; Fisher et al. 1975; Austin and Krauss 1986; Austin et al. 1988*b*, 1990*a*, 1990*b*). Recent results

Table 8

Observed Segregation Ratios of LDL Subclass Patterns A ($K \geq 0$) and B ($K < 0$)

Mating Type (no. of matings)	No. (%) of Offspring in		
	Pattern A	Pattern B	Total
A × A (2)	17 (94%)	1 (6%)	18 (100%)
A × B (12)	42 (62%)	26 (38%)	68 (100%)
B × B (5)	14 (40%)	21 (60%)	35 (100%)

from Austin et al.'s (1988*b*) study of Mormons living in California are similar to our findings among Dutch families, in indicating that a major locus is involved in determining the LDL subfraction pattern distribution (Austin et al. 1988*b*). However, our data suggest that the inherited major gene was more likely to be recessive than dominant, whereas among the Mormon families a dominant mode of inheritance for the major locus was reported. It should be noted, however, that both the recessive and additive major-locus models could not be strongly rejected by Austin et al. (1988*b*) ($.05<P<.1$).

In addition, we now report a significant multifactorial inheritance component in the distribution of the LDL subfraction profile among the Dutch families, whereas no multifactorial heritability was found in the Mormon data. Possibly, differences in prevalences of nongenetic risk factors—e.g., smoking and drinking habits, diet, relative body weight, and oral contraceptive use—may in part explain the apparent differences in genetic models, as these environmental influences have been associated with the LDL subfraction pattern (Terry et al. 1985; Swinkels et al. 1989*a*; de Graaf et al. 1991*b*). The presence, in the present study, of several of these environmental risk factors, along with the high prevalence of a dense LDL subfraction pattern, provided an opportunity to assess both environmental and genetic influences in the etiology of the LDL subfraction pattern distribution in the Dutch population. The exclusion of most environmental risk factors among the Mormons, because of their life style, may explain why no multifactorial inheritance component was found in data on them (Austin et al. 1988*b*).

Furthermore, the inheritance of the LDL subfraction patterns in the present report is based on the density distribution of the LDL particles, as LDL subfractions were detected by a density gradient ultracentrifugation method. Austin et al. (1988*b*) detected LDL subfractions by gradient gel electrophoresis, which separates the LDL particles on the basis of the difference in their sizes. These two properties of the LDL subfractions—i.e., the size and the density—could differ in their susceptibility to genetic influences, contributing to a different mode of inheritance.

The differences in genetic models also raises the question whether the same alleles for inherited susceptibility occur in the two populations and whether gene dosage and gene-environment interaction differ between Mormons and the Dutch population. However, among American Caucasian subjects with familial combined hyperlipidemia, the LDL subfraction pattern, isolated by gradient gel electrophoresis, appeared to be influenced also by a major locus with an additive mode of inheritance and a significant multifactorial component (Austin et al. 1990*a*), suggesting that the pattern of inheritance of the LDL subfraction profile is similar among different populations.

In the present study, using density gradient ultracentrifugation, it appeared that each subject showed his or her own specific LDL subfraction pattern (Swinkels et al. 1989*b*). To reflect the great interindividual variability in LDL subfraction profile, the LDL subfraction pattern was considered a quantitative trait and was approached by the continuous parameter K, which allows the contribution of all three major LDL subfractions to be taken into account. Most reports on LDL subfractions distinguish only two distinct LDL subfraction patterns, denoted "A" and "B," on the basis of the size of the LDL particle of only the major LDL subfraction (McNamara et al. 1987; Austin et al. 1988*a*, 1988*b*; Campos et al. 1988). To be able to compare our results with those in the literature, we also defined a pattern A/pattern B classification by defining a threshold for parameter K. The distribution of LDL subfraction pattern A/B determined on the basis of parameter K, was similar to that described by Austin et al. and McNamara et al., who defined their pattern A/B classification on the basis of the size of the major LDL subfraction (table 1). These close similarities in the distribution of pattern A/B, under different definitions, indicate a strong relationship between K and the A/B classification, as reported by others.

When a quantitative trait such as the LDL subfraction pattern is reduced to a dichotomy—i.e., LDL subfraction patterns A and B—much information is lost, since we do not know whether an individual is close to or far from the threshold. Moreover, where familial data are useful, quantitative traits of relatives are often more informative than their affection status (Morton and MacLean 1974). In addition, the mixed model is originally formulated in terms of quantitative traits. When, under a dichotomous classification, the mixed model is applied—by defining, on an underlying continuous-liability scale, a threshold whose crossing results in affection—several difficulties have been reported (Vogler et al. 1990; for example, there can be flatness of the likelihood surface, which complicates maximization of the likelihood, or local maxima obscuring true maximum likelihood. Indeed, in the present report, when the dichotomous classification of the

LDL subfraction—i.e., into patterns A and B—was used, the results of the segregation analysis suggested inheritance of the LDL subfraction pattern; but a more specific model of inheritance could not be defined. Most likely, since there is less power in using a dichotomous trait for segregation analysis, the sample size in the present study was not sufficiently large to discriminate among complex models of inheritance. Similarly, the discrimination among the Mendelian single-locus models reported by Austin et al. (1988*b*) was not strong. However, application of the quantitative parameter K in the segregation analysis resulted in a higher power to discriminate among complex hypotheses, compared with the pattern A/pattern B classification; when using parameter K, a clear model of inheritance, the general mixed model was the only model that fitted the data; all the other models tested were strongly rejected ($P < .001$). Our results thus suggest that the quantitative variable, parameter K, provides more information, resulting in the segregation analysis having a higher power to discriminate between complex models of inheritance. For the putative major locus, the penetrance of the more dense LDL subfraction profiles ($K<0$) for subjects with genotype Cc was remarkably high and dependent on gender, age, and (in women) hormonal status. Even in the absence of the defective c allele (genotype CC), expression of dense LDL subfraction patterns ($K<0$) was expected (table 6). These results indicate that the phenotypic expression of the major locus responsible for dense LDL subfraction patterns ($K<0$) is strongly modulated by environmental, behavioral, and/or genetic background. In the general mixed model, 64% of the total variance in transmission of the heavy LDL subfraction patterns was represented by the major locus, whereas 11% was accounted for by multifactorial inheritance and 25% by random environmental influences. These results support the hypothesis of etiological (i.e., transmission) heterogeneity, whereby in some families the dense LDL subfractions are primarily due to a major gene, whereas in other families the disease could be accounted for mainly by the multifactorial inheritance component. This implies that both a major gene and environmental influences are operating, to variable extents, in the families studied.

In an attempt to differentiate between genetically determined and environmental influences, the effects of smoking, body weight, OC use, and lipid and lipoprotein levels on the variability in LDL subfraction pattern were evaluated. In the present study, it appeared that 60% of the variation in parameter K—i.e., the LDL subfraction pattern—could be explained by gender, age, BMI, smoking habits, hormonal status in women (20%), and lipid and lipoprotein levels (40%), confirming the results of previous reports (McNamara et al. 1987; Swinkels et al. 1989*b*). Also, other biochemical influences that may be involved in the generation of LDL subfractions, such as the activity of several enzymes (cholesteryl ester transfer protein [Gambert et al. 1990], hepatic lipase [Auwerx et al. 1989], and lipoprotein lipase [Chait et al. 1984]) must be considered to account for some of the variability in the LDL subfraction distribution.

A dense LDL subfraction pattern, characterized by a high prevalence of small, dense LDL particles, has been associated with a high-risk lipoprotein profile, reflected by increased levels of serum triglycerides and decreased HDL-cholesterol levels (McNamara et al. 1987; Swinkels et al. 1989*b*; Austin et al. 1990*b*; Campos et al. 1992). This strong association between a dense LDL subfraction pattern and an atherogenic lipid profile raises the question whether the proposed gene for the dense LDL subfraction pattern is also responsible for the associated lipid and lipoprotein levels. Alternatively, other genetic and environmental factors could influence the lipid and lipoprotein levels (Segal et al. 1982), which in turn may contribute to a heavy LDL subfraction pattern. Further elucidation of the genes involved in the expression of the LDL subfraction pattern and in lipid and lipoprotein levels could help us to understand these complex interrelationships.

In summary, we conclude that the LDL subfraction pattern is a quantitative trait that can be described using the continuous parameter K. Application of parameter K in the segregation analysis resulted in a higher power to discriminate between complex modes of inheritance, compared with the dichotomous classification into a light and a heavy LDL subfraction pattern. The results indicate that the distribution of parameter K—i.e., the LDL subfraction pattern in Dutch families—is the result of a combination of underlying genetic traits and environmental or behavioral traits. Mapping and molecular characterization of this inherited susceptibility could both substantially aid the understanding of the prevalence of the dense LDL subfraction profile and contribute to the explanation of familial aggregation of CHD.

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